International Journal of Drug Delivery 5 (2013) 146-151

http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

Determination of bosentan in pharmaceutical dosage forms by high performance liquid chromatography

Petikam Lavudu[1], Avula Prameela Rani[2], Are Purna Chander[3], Chandra Bala Sekaran[4*]

*Corresponding author:

Chandra Bala Sekaran

[1]Department of Pharmaceutical Biotechnology, Vishnu Institute of Pharmaceutical Education and Research, Narsapur, A P- India.
[2]University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Guntur, A P-India
[3]Department of Pharmacology, Vishnu Institute of Pharmaceutical Education and Research, Narsapur, A P- India.
[4]Department of Biotechnology, Jagarlamudi Kuppuswamy Choudary College, Guntur, A P-India.

Abstract

A simple and sensitive high performance liquid chromatographic method is developed for the estimation of bosentan in pharmaceutical dosage forms. Chromatographic separation of the drug was achieved with an Thermo Scientific C18 column (250 mm 4.6 mm I.D., 5 µm particle size) analytical column using ammonium bicarbonate (pH was adjusted to 5.0 with phosphoric acid) and acetonitrile (70:30 *v/v*) as mobile phase. The instrumental settings are flow rate of 1.0 ml/min, column temperature at 25±1 C, detector wavelength of 220 nm and the run time was 5 min. The retention time of the drug was 1.986 min. The developed method shows linearity over a range of 5-100 µg/ml of bosentan with correlation coefficient of 0.9991. The relative standard deviation is less than 1.5%. The proposed method was found to be suitable and accurate for quantitative determination of bosentan in pharmaceutical dosage forms.

Keywords: HPLC; Bosentan; Ammonium bicarbonate; Acetonitrile; Dosage forms.

Introduction

Pulmonary arterial hypertension (PAH) is a chronic, life-threatening disorder which severely hampers the function of the lungs and heart. In individuals with PAH, the levels of endothelin which is a potent endogenous vasoconstrictor is increased. Bosentan (BSN) [1-4] has been prescribed for the management of PAH. BSN is a vasodilator and oral dual endothelin receptor antagonist. BSN acts by competitively antagonizing the binding of endothelin to both endothelin receptors, ET_A and ET_B. Chemically, BSN is known as N-[6-(2-Hydroxyethoxy)-5-(2-methoxyphenoxy)-2-pyrimidin-2-yl-pyrimidin-4-yl]-4-tert-butyl-benzenesulfonamide (Figure 1).

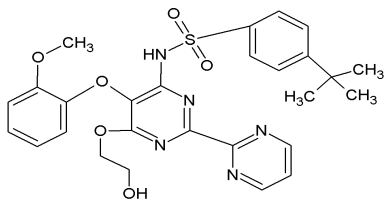

Figure 1. Structure of bosentan

Several analytical methods have been described in the literature for the determination of BSN in pharmaceutical dosage forms and biological fluids. UV-spectrophotometric methods [5-8] employing different solvents have been reported for the assay of BSN in pharmaceutical dosage forms. These methods suffered from the lack of selectivity and sensitivity.

Khan et al. [9] developed a stability indicating HPLC method for the determination of related substances of BSN. Jadhav et al. [10] proposed a stability indicating gradient reverse phase liquid chromatographic method for the determination of process and degradation impurities in BSN. The reported stability indicating methods are sensitive but suffered from disadvantages like having a narrow range of linear response, more retention time and lesser precision. Further more these methods are not applied to pharmaceutical dosage forms.

Two narrow-bore liquid chromatography with ion spray tandem mass spectrometric detection methods (LC-MS) were reported by Lausecker et al. [11,12] The first method is applied for the determination of BSN in human plasma whereas the second method is useful for simultaneous determination of BSN and its three main metabolites in plasma, serum, bile, and liver samples from man, dog and rat. The reported LC-MS methods require expensive detector, tedious sample preparation steps, require a

skilled person to operate the instrument. These features make them unattractive to routine analysis.

There are few reports on the application of HPLC with UV detection [13-15] for the assay of BSN in bulk drug and pharmaceutical dosage forms. The disadvantages of the reported HPLC with UV detection methods are lack of sensitivity, lesser precision and having a narrow range of linear response. In addition, the retention time of the BSN is more which leads to a longer runtime for a single sample.

The aim of the present study is to develop and validate a simple, fast, sensitive and precise HPLC with UV detection method for the assay of BSN in bulk and in pharmaceutical dosage forms.

Materials and Methods

Apparatus

Chromatographic data were obtained using Shimadzu HPLC class VP series, (Shimadzu Corporation, Kyoto, Japan) isocratic high pressure liquid chromatographic system equipped with an LC-10 AT pump, a variable wavelength programmable UV/Visible detector SPD-10A, a CTO-10 AS column oven, an SCL-10A system controller. The chromatographic system utilizes a Shimadzu class VP series version 5.03 computer program to control hardware, and to acquire and store data.

Thermo Scientific C18 column (250 mm 4.6 mm I.D., 5 μm particle size, Phenomenex, Torrance, CA, USA) was used for the separation of BSN.

Shimazdu (Tokyo, Japan) electronic weighing balance, model BL 220 H was used for weighing the samples.

Elico pH meter (Hyderabad, India) LI 120 model was used for pH measurements.

Chemicals

Milli-Q-water was used all the way through the process. It was obtained from Merck Specialties Private Ltd, Hyderabad, India. HPLC grade quality acetonitrile was purchased from Rankem laboratories, Mumbai, India. HPLC grade quality orthophosphoric acid and ammonium bicarbonate were purchased from Merck, Mumbai, India. Bosentan reference standard was kindly supplied by MSN laboratories, Hyderabad. Bosentas tablets (*Cipla Private Limited, Hyderabad, India)*, labeled to contain 62.5 and 125 mg bosentan per tablet, were purchased from commercial sources in the local pharmacy market.

Mobile Phase

The mobile phase is composed of a mixture of 20 mM ammonium bicarbonate (pH 5.0) and acetonitrile in the ratio of 70:30 (*v/v*). Ammonium bicarbonate (20 mM) was prepared by dissolving 0.158 gm of ammonium bicarbonate in 100 ml of Milli-Q-water and the pH was adjusted to 5.0 with orthophosphoric acid. The mobile phase was filtered through a 0.45 μm membrane filter and degassed with a helium sparge for 15 min prior to use.

Standard Drug Solutions

The standard stock solution of BSN (1 mg/ml) was prepared in mobile phase. The working standard solutions (5, 10, 20, 40, 60, 80, 100 μg/ml) were prepared by appropriate dilution of the stock solution with mobile phase.

Chromatographic Conditions

Column	:	Thermo Scientific C18 column
Mode	:	Reverse phase
Mobile phase	:	20 mM Ammonium bicarbonate (pH5.0): acetonitrile (70:30 *v/v*)
Flow rate	:	1 ml/min
Column temperature	:	25±1 C
Injection volume	:	20 μl
Detection wavelength	:	220 nm
Runtime	:	5 min

Construction of the Calibration Graph

Working standard solutions containing 5, 10, 20, 40, 60, 80, 100 μg/ml of BSN were prepared by the dilution of standard stock BSN solution with the mobile phase. The mobile phase was pumped for about 30 minutes to saturate the column thereby to get the base line corrected. Twenty μL aliquot of each concentration was injected (triplicate) and eluted with the mobile phase under the chromatographic conditions described above. The average peak areas of BSN were plotted versus the final concentration of the BSN in μg/ml to get the calibration graph. Alternatively, the corresponding regression equation was derived. The concentration of the BSN was calculated either from the calibration graph or from the regression equation.

Analysis of Bosentas tablets

Ten Bosentas tablets were accurately weighed, finely crushed and thoroughly mixed. A quantity of the powder equivalent to 50 mg of BSN was transferred into a small conical flask and extracted with 20 ml of mobile phase. The extract was filtered through 0.45 mm membrane filter into a 50 ml volumetric flask. The conical flask was washed with few ml of mobile phase and the washings were passed into the same volumetric flask and completed to the volume with the mobile phase. This solution was further diluted suitably with the same solvent to get 50 μg/ml of BSN. Twenty μL of the above solution was injected (triplicate) and eluted with the mobile phase under the chromatographic conditions described above. The nominal content of BSN in the tablets was computed either from the previously plotted calibration graph or from the regression equation.

Results and Discussion

Method Development

In order to get the enhanced efficiency of the chromatographic system, the experimental conditions such as column, column temperature, mobile phase composition, pH of mobile phase and detection wavelength were optimized by varying one parameter at

a time and keeping the others constant. The results of optimization experiments have revealed that the Thermo Scientific C18 column (250 mm 4.6 mm I.D., 5 µm particle size) maintained at ambient temperature was suitable for the separation of BSN. Concerning the mobile phase, a mixture of 20 mM ammonium bicarbonate and acetonitrile was used. In order to get better separation and peak symmetry, the composition and pH of the mobile phase was varied until optimum composition and pH was selected. The best result was obtained by use of 70:30 (*v/v*) ratio of 20 mM ammonium bicarbonate and acetonitrile (pH 5.0 ± 0.05 adjusted with orthophosphoric acid). Isocratic elution at flow rate of 1.0 ml/min has been employed in the present study. Wavelength of 220 nm was selected for the UV detection. Under the optimum chromatographic conditions, the retention time obtained for BSN was 1.986 min.

Method validation

The proposed HPLC method was validated for parameters, such as system suitability, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and robustness, according to International Conference on Harmonization (ICH) guidelines [16].

The system suitability was studied by performing the experiment and looking for changes in retention time, peak area and peak asymmetry. Five injections of the standard solution of BSN (20 µg/ml) were injected for this purpose. The system suitability was confirmed by calculating the relative standard deviation values for parameters like retention time, peak area, peak asymmetry, theoretical plates, plates per meter and height equivalent to theoretical plate. It was observed that all the values are within the limits (Table 1).

Table 1. System suitability parameters of bosentan

Parameter	Value*	%RSD
Retention time (t) (Min)	1.986	0.25
Peak area	932524	0.10
Theoretical Plates (n)	4816	0.62
Plates per Meter (N)	19264	1.10
Height equivalent to theoretical plate(HETP) (mm)	5.2×10^{-7}	1.05
Peak asymmetry	0.98	1.02

* Average of five values

The specificity study was carried out to confirm the absence of interference by the excipients in the tablet and components of mobile phase. Specificity of the proposed method is evaluated by comparing the chromatograms of tablet sample, blank mobile phase and standard BSN. It was observed that none of the peaks appears at the same retention time of BSN (Figures 2, 3 and 4). This confirmed the specificity of the method.

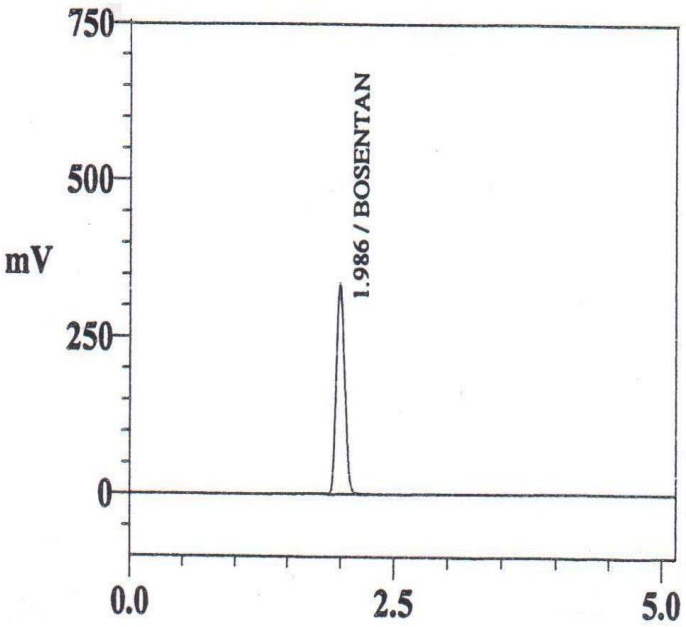

Figure 2. Chromatogram of standard bosentan (40 µg/ml)

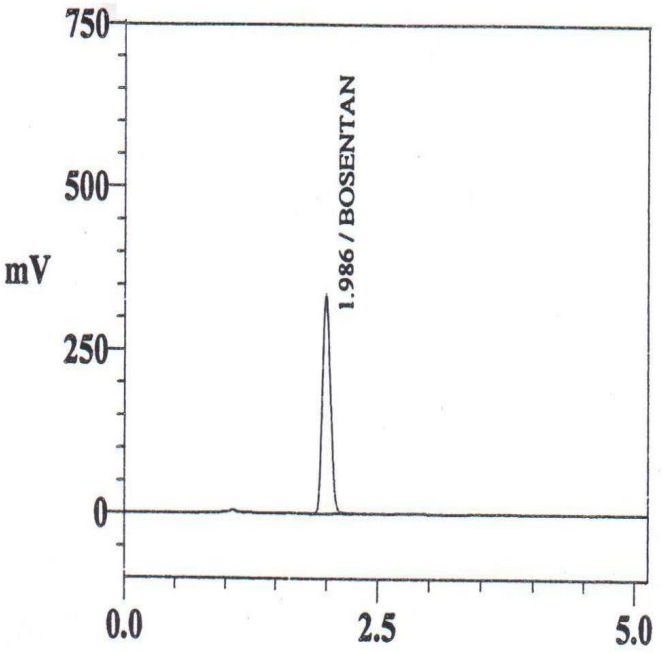

Figure 3. Chromatogram of bosentan tablet sample (40 g/ml)

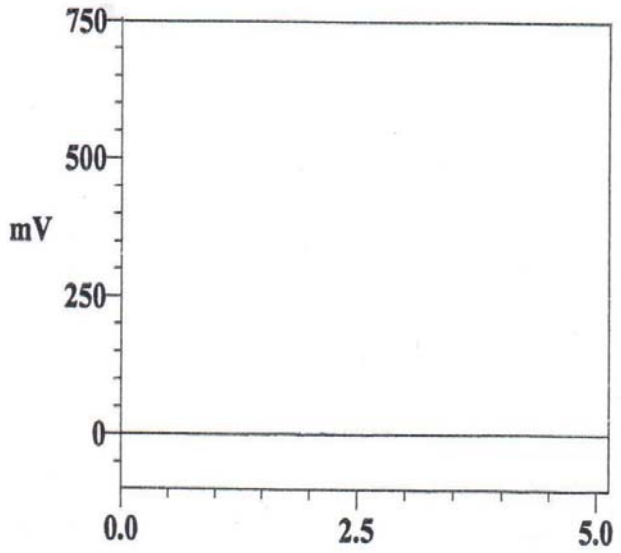

Figure 4. Chromatogram of blank mobile phase

To detect the linearity of the proposed method, seven working standard solutions of BSN (5, 10, 20, 40, 60, 80, 100 μg/ml) were injected in to the column in triplicate. The chromatograms were recorded. Linearity was observed by plotting drug concentration against peak areas. BSN showed linear response in the concentration range of 5–100 μg/ml. Linear regression analysis of the concentration-peak area data gave the following equation:

$$Y = 47752x - 4217.1;\ R^2 = 0.9991$$

Where 'x' is the concentration of BSN in μg/ml, 'Y' is the peak area and 'R^2' is the regression coefficient showing excellent linearity of the proposed method.

In order to determine limit of detection (LOD) and limit of quantification (LOQ), concentration in the lower part of the linear range (5 μg/ml) of the calibration graph was used. A solution of BSN (5 μg/ml) was prepared and injected into the system (n=5). The standard deviation (SD) of responses was calculated. Limit of detection was calculated by (3.3 SD)/m and limit of quantification was calculated by (10 SD)/m, where "m" corresponds to the slope obtained in the linearity study. The calculated LOD and LOQ values were 0.225 and 0.684 μg/ml, respectively. This indicates the adequate sensitivity of the method.

The precision and accuracy of the proposed method was determined by intra-day and inter-day analysis. Intra-day analysis was carried out by performing five repeated analysis of BSN at three different concentrations levels (5, 50 and 100 μg/ml) on the same day, under the same experimental conditions. The inter-day analysis of the method was assessed by carrying out the analysis for three consecutive days (inter-day). The results are given in Table 2. The standard deviation, relative standard deviation and recoveries obtained for the proposed method can be considered to be satisfactory.

Table 2. Precision and accuracy of the proposed method

Concentration of BSN (μg/ml)		% Recovery	% RSD
Taken	Found ± S.D*		
Intra-day analysis			
5	4.98 ± 0.016	99.92	0.321
50	50.02 ± 0.121	100.06	0.241
100	99.94 ± 0.336	99.94	0.336
Inter-day analysis			
5	4.97 ± 0.021	99.40	0.422
50	49.98 ± 0.161	99.96	0.322
100	100.06± 0.428	100.06	0.427

* Average of five determinations

The accuracy of the proposed method was also established by carrying out recovery experiments through standard addition technique. For this purpose, pure BSN was spiked to the pre-analyzed formulation. The total amount of BSN was once again found by the proposed method. The recovery of the BSN was calculated. The results are presented in Table 3. The results showed that the common excipients present in tablets did not interfere in the assay of BSN by the proposed method. Thus the proposed method is very efficient for the assay of BSN.

Table 3. Recovery study standard addition technique

Label claim (mg/tablet)	Pure BSN spiked (mg)	Found (mg) ± S.D*	Recovery (%)
62.5	31.25	93.74 ± 0.842	99.89
125.0	62.50	187.48 ±1.808	99.96

* Average of three determinations

The robustness of the proposed method was examined by deliberately changing chromatographic parameters such as mobile phase composition, flow rate, pH of the mobile phase and detection wavelength. For this study, three consecutive injections of BSN standard solutions (5 and 100 μg/ml) were applied. The results are summarized in Table 4. It is obvious from the results that none of these variables significantly affected the assay of BSN indicating that the proposed method could be considered as robust.

Table 4. Robustness of the proposed method

Variable	Concentration of BSN (μg/ml)		% Recovery	% RSD
	Taken	Found ± S.D*		
Mobile phase (70:30 ± 2%)	5	5.02 ± 0.020	100.40	0.398
	100	99.96± 0.141	99.96	0.141
pH of the mobile phase (5±0.2)	5	4.99 ± 0.033	99.80	0.661
	100	99.78 ± 0.221	99.78	0.221
Flow rate (1 ± 0.1 ml/min)	5	4.88 ± 0.015	97.60	0.307
	100	99.76± 0.321	99.76	0.321
Detection wavelength (220 ± 1 nm)	5	5.02 ± 0.029	100.04	0.577
	100	99.66± 0.161	99.66	0.161

* Average of three determinations

Application of the method to tablet dosage forms

The proposed method was applied for the estimation of BSN in tablet dosage form. The results are shown in Table 5. The high recovery with low RSD value confirms the appropriateness of the proposed method for the routine analysis of BSN in tablet dosage forms.

Table 5. Analysis of tablet dosage forms by the proposed method

Tablet brand name	Label claim (mg/tablet)	Found (mg) ± S.D*	RSD (%)	Recovery (%)
Bosentas	62.50	61.49 ± 0.501	0.814	99.66
	125.00	124.88±0.998	0.799	99.96

* Average of three determinations

Conclusion

A reversed phase HPLC with UV detection method was developed for the determination of BSN in bulk and tablet dosage forms. The method was validated according to the International Conference on Harmonization guidelines. The method was found to be simple, economical, precise, accurate, specific, sensitive and robust. The features like short retention time (1.99 min), runtime (5 min), and flow rate (1 ml/min) make the method more attractive. These features help in decreasing the cost and time of analysis. The results of the analysis of tablet dosage forms are highly reproducible and are in good agreement with the label claim of the drug. The excipients generally present in the pharmaceutical formulations do not interfere in the assay of BSN. Hence, the developed method can be easily and conveniently adopted for routine analysis of BSN in quality control laboratories.

Acknowledgements

One of the authors, P. Lavudu, expresses his gratitude to Dr. Alluri Ramesh, principal, Vishnu Institute of Pharmaceutical Education & Research, Narsapur and University College of Pharmaceutical sciences, Acharya Nagarjuna University, Guntur for providing research facilities.

Competing interests

The authors did not have any competing interests.

.

References

[1]. Raja SG, Dreyfus GD. Current status of bosentan for treatment of pulmonary hypertension. Ann. Card. Anaesth. 2008; 11(1): 6-14.

[2]. Gabbay E, Fraser J, McNeil K. Review of bosentan in the management of pulmonary arterial hypertension. Vasc. Health. Risk. Manag. 2007; 3(6): 887–900.

[3]. Roux S, Breu V, Ertel SI, Clozel M. Endothelin antagonism with bosentan: A review of potential applications. J. Mol. Med.1999; 77(4): 364-376.

[4]. Dingemanse J, Van Giersbergen PL. Clinical pharmacology of bosentan: A dual endothelin receptor antagonist. Clin. Pharmacokinet. 2004; 43(15): 1089-1115.

[5]. Annapurna MM, Bisht SPS, Kumar BVVR, Kumar VR, Narendra A. Spectrophotometric determination of bosentan and its application in pharmaceutical analysis. Int. J. Comp. Pharma. 2011; 2 (1): 1-2.

[6]. Ashok Kumar A, Anil Kumar A, Sankar DG. Development, estimation and validation of bosentan in bulk and in its pharmaceutical formulation by UV-VIS spectroscopic method. Int. J. Pharma. Bio. Sci. 2011; 2(2): 225-230.

[7]. Kumar D, Sreenivas SA, Samal HB, Dey S, Priyanka Y. Method development and estimation of bosentan monohydrate in bulk and pharmaceutical dosage forms using UV-Visible spectrophotometer. J. Pharma. Res. 2011; 4(6): 1713-1715.

[8]. Narendra A, Deepika D, Annapurna MM. New spectrophotometric method for the determination of bosentan - An anti-hypertensive agent in pharmaceutical dosage forms. E-J. Chem. 2012; 9(2): 700-704.

[9]. Khan MA, Sinha S, Todkar M, Parashar V, Swamy K. Development and validation of a stability indicating analytical method for the related substances of bosentan drug substance by HPLC. American J. Sci. Ind. Res. 2012; 3(2): 69-80.

[10]. Jadhav SA, Landge SB, Jadhav SL, Niphade NC, Bembalkar SR, Mathad VT. Stability-indicating gradient RP-LC method for the determination of process and degradation impurities in bosentan monohydrate: An endothelin receptor antagonist. Chromatogr. Res. Int. 2011; 2011(ID 929876): 1-5.

[11]. Lausecker B, Hopfgartner G. Determination of an endothelin receptor antagonist in human plasma by narrow-bore liquid chromatography and ion spray tandem massspectrometry. J. Chromatogr. A. 1995; 712(1): 75-83.

[12]. Lausecker B, Hess B, Fischer G, Mueller M, Hopfgartner G. Simultaneous determination of bosentan and its three major metabolites in various biological matrices and species using narrow bore liquid chromatography with ion spray tandem mass spectrometric detection. J. Chromatogr. B. Biomed. Sci. Appl. 2000; 749(1): 67-83.

[13]. Muralidharan S, Kumar JR. Simple estimation of bosentan in tablet formulation by RP-HPLC. American J. Anal. Chem. 2012; 3(11): 715-718.

[14]. Reddy TK, Younus Md, Reddy YR, Kumar GA, Sravan S. RP-HPLC method development and validation of bosentan

drug present in tablets. Int. J. Pharma. Tech. 2010; 2(3): 577-587.

[15]. Rao TN, Patrudu TB, Raghubabu K, Nagachandrudu S, Sreenivasulu D, Reddy EGS. Estimation of bosentan monohydrate in tablet dosage forms by a new RP-HPLC method. Am. J. PharmTech Res. 2012; 2(5): 391-398.

[16]. Validation of Analytical Procedures; Methodology, International Conference on Harmonization (ICH), Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London.